The effects of early experience on habitat selection in tadpoles of the Malayan painted frog, *Kaloula pulchra* (Anura : Microhylidae)[1]

Fred Punzo[2]
Dept. of Zoology, Iowa State University, Ames, Iowa 50010 (*USA*)
(*With two text-figures*)

In this study, the effects of early experience on subsequent habitat selection by tadpoles of the Malayan painted frog, *Kaloula pulchra* (Anura: Microhylidae) were investigated. Tadpoles from laboratory-hatched eggs were reared on various artificial substrate patterns. Animals reared in white trays (featureless environment) exhibited no habitat preferences. Tadpoles reared in a stripe-patterned environment showed a marked preference for the striped substrate area of the test chamber. Similarly, tadpoles reared in a square-patterned habitat exhibited a strong preference for the squared substrate when given a choice between squared and striped substrate pattern types. It was also demonstrated that once a habitat preference had been established, it was retained even though a period of isolation from the original rearing substrate pattern. The results of these experiments indicate that larval amphibians can learn to respond to physical features of their environment and associate habitat preference responses with these features. The adaptive significance of early experience effects on habitat selection are also discussed.

Introduction

It is a well established fact that habitat selection in vertebrates is predicated upon some active response to one or more specific stimuli present in the environment (Heatwole 1961; Hilden 1965; Wiens 1970). These stimuli are frequently associated with the spatial patterning of the habitat (Klopfer & Hailman 1965). In recent years there has been an emphasis placed on the behavioural mechanisms involved in habitat selection responses, and it has been demonstrated that the effects of early experience can have significant effects upon the subsequent responses made by the organism to physiognomic features of the environment (Sargent 1965; Wecker 1963; Wiens 1972). The majority of previous studies have concentrated on mammals (Ambrose 1973; Barash 1973; Cameron & Rainey 1972; Geluso 1971; Miller 1942; Wecker 1963) and birds (Hilden 1965; Klopfer 1967; Lack & Venables 1939; Sargent 1965). With respect to amphibians and reptiles, most of the studies have focused on the physical features of the habitat (Goodman & Goin 1970; Heatwole 1962), while relatively few studies have concerned themselves

[1] Accepted February 1975.
[2] *Present address*: Dept. of Biology, Blackburn College, Carlinville, Illinois 62626, U.S.A.

with the processes involved in habitat selection (Heatwole 1961; McKenzie & Storm 1970; Sexton & Ortleb 1966; Wiens 1970, 1972). Prior investigations have demonstrated that adult amphibians do respond selectively to various physical features of the habitat (Sexton *et al.* 1964). However, the role of larval experience in the ontogeny of habitat preferences is not completely understood. In addition, there have been relatively few studies on the relationship between learning and retention capacities of amphibians (Chu & McCain 1969; Kuntz 1923; Munn 1940; Noble 1931; Schneider 1968; Sluckin 1965; Thorpe 1963) and their possible adaptive significance with respect to habitat selection behaviour.

In the present study, the effects of early experience on subsequent habitat selection by tadpoles of the Malayan painted frog, *Kaloula pulchra,* were investigated, as well as the retention capacities of this amphibian.

Materials and Methods of study

The *Kaloula pulchra* tadpoles utilized in this study were obtained from breeding adults in the laboratory. Mature males and females were allowed to breed and the fertilized eggs were placed in plexiglass trays containing distilled water maintained at 22°C. Immediately upon hatching, the tadpoles were individually isolated and placed in white porcelain trays. They were kept under a standard photoperiod interval (16L: 8D). The tadpoles were maintained in this manner for a period of one week after which they were divided into four groups of 90 individuals and subjected to different rearing substrate patterns for a period of two weeks. The test animals were fed on a diet of rabbit pellets.

Control group tadpoles (Group 1) were reared in a relatively sterile environment devoid of any patterning. They were kept in white trays (50 × 35 × 10 cm) throughout the experiment and were maintained in this manner at 22°C and a photoperiod of 16L: 8D.

Group 2 tadpoles were subdivided into two groups, each reared in one of two experimental habitats throughout their development. These habitats consisted of enclosed chambers (70 × 110 cm) provided with fluorescent lighting and a one-way viewing glass. Substrate patterns were produced by using black plastic tape against the white background floor of the rearing trays. One pattern consisted of black parallel stripes 2.5 cm wide and 2.5 cm apart; the other substrate pattern consisted of black squares (2.5 × 2.5 cm) arranged in a linear sequence, 2.5 cm apart.

Group 3 tadpoles were tested for two weeks as those in Group 2 and then transferred to sterile chambers devoid of patterning and similar to those of the control group for a period of one week.

In order to ascertain the effects of early experience on subsequent habitat selection, individual animals were tested for preference between the two substrate patterns discussed above. The tadpoles were placed in an enclosed testing chamber (70 × 70 cm) provided with a one-way viewing glass. Temperature, water and light regimes were identical to rearing conditions. The floor and sides of half of the test chamber were provided with a striped pattern identical to that used in the rearing habitat, while the other half of the chamber was covered with black squares (Fig. 1). A transparent plexiglass tube, 5 cm in diameter and open to both ends, was placed vertically in the centre of the chamber directly over the boundary between the two substrate patterns (Fig. 1, X). The test chamber was filled with distilled water to a depth of 8 cm.

For each trial, the tadpole to be tested was removed from its experimental habitat, placed in the vertical tube and then released into the test chamber. All experimental animals exhibited some preliminary exploratory activity followed by definitive orientation movements toward a specific substrate pattern. The choice of substrate pattern as well as the amount of time spent on each pattern during a 3-minute interval following placement in the vertical

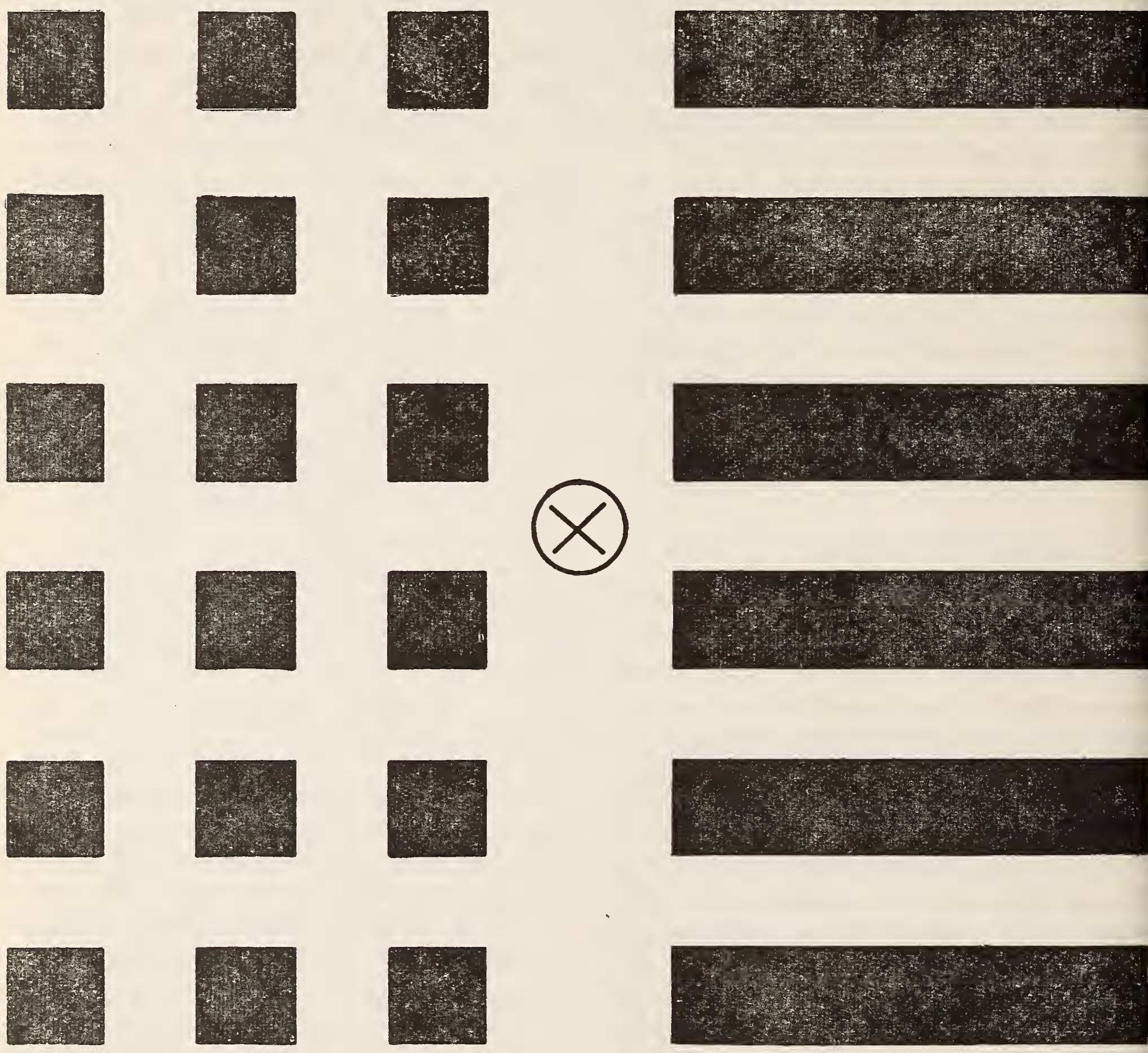

Fig. 1. Diagrammatic representation of the floor of the test chamber showing the two types of experimental substrate pattern types. (X) refers to the vertical tube placed at the boundary between the squared and striped substrate patterns.

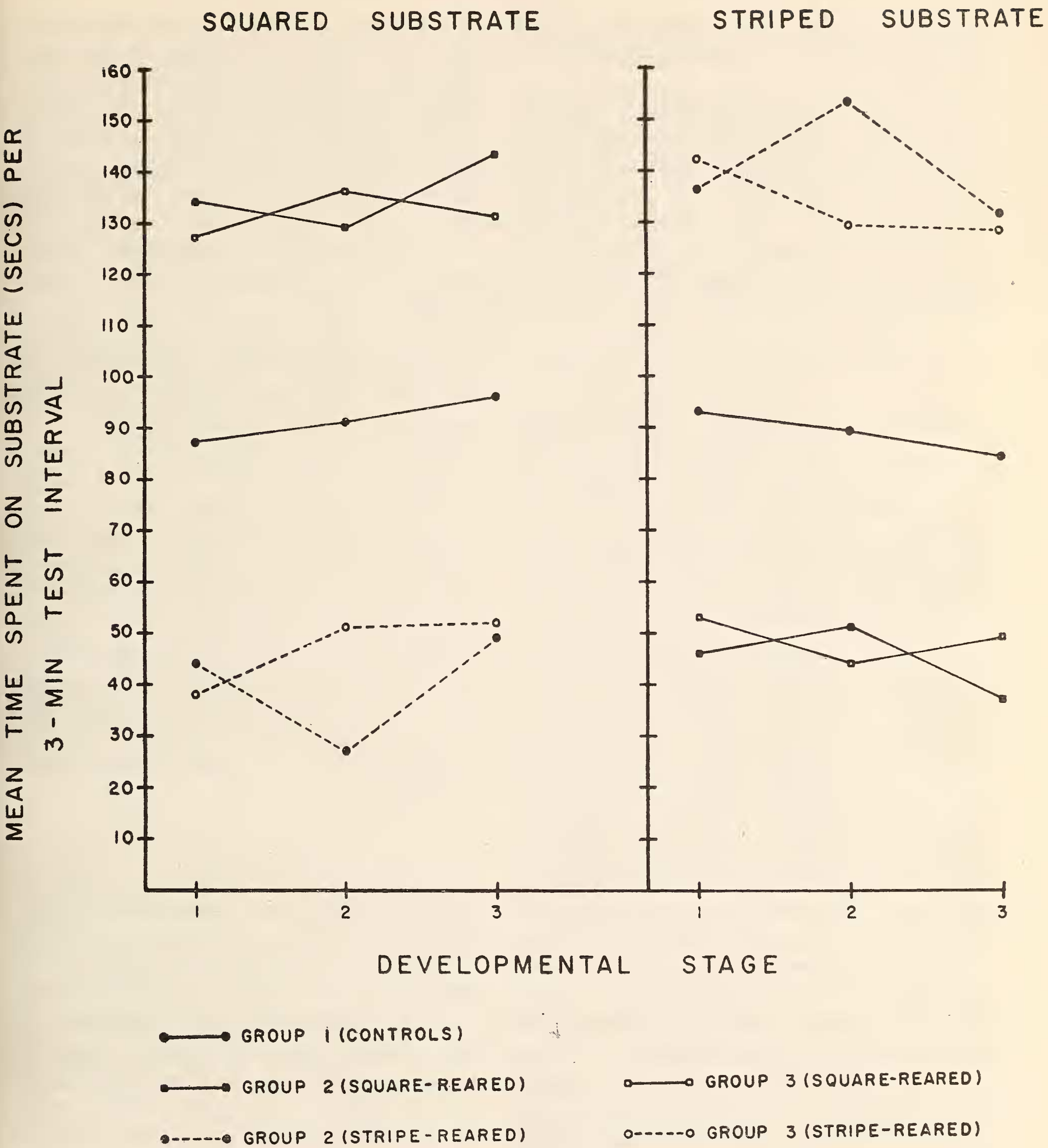

Fig. 2. Mean time in seconds spent on each substrate pattern per 3-minute test interval.

tube were recorded. Upon termination of test trials the tadpoles were returned to their rearing habitats.

Pattern preferences were analyzed at three developmental stages based on larval length in mm (Gosner 1960): (1) 10-15 mm (young tadpoles with no visible hind leg development); (2) 15-25 mm (tadpoles exhibiting hind leg development); (3) 25-35 mm (metamorphosis essentially complete with only a small remnant of the larval tail remaining).

Results

Choice of substrate pattern (Table 1) and time spent on each substrate (Fig. 2) were found to be significant indices of habitat preferences. Control animals (Group 1) showed no preference for either substrate pattern, and all three developmental stages spent fairly equal amounts of time on both patterns.

Group 2 tadpoles that were reared in a square-patterned habitat exhibited a marked preference for the square pattern area of the test chamber, both in their initial choice (Table 1) and in the amount of time spent in the square versus the stripe-patterned habitat (Fig. 2). Animals reared in a stripe-patterned habitat similarly showed a preference for the striped substrate area of the test chamber. This suggests that such preferences were established during the initial rearing periods in the squared and striped experimental habitats. Qualitative observations verify the biological significance of the above results. At all three developmental stages, the tadpoles that were reared in the squared habitat and selected the squared substrate pattern when tested, swam vigorously during the 3-minute test period. Frequently, some individuals would swim toward the boundary between the two substrate patterns. Upon reaching this boundary the tadpoles would suddenly stop and terminate locomotor activity for several seconds. After this short pause, the animals would either dart back into the squared substrate area or swim parallel to the boundary within the squared area of the test chamber. Likewise, animals reared in the striped habitat would approach the boundary within the striped section of the chamber. Occasionally, square-reared tadpoles would venture into the striped substrate area but would remain there for only brief periods and then rapidly return to the squared area. Similar observations were noted for stripe-reared tadpoles that infrequently would enter the squared area.

Group 3 tadpoles were used to ascertain whether or not an initial substrate pattern preference could be retained over a period of isolation from the rearing substrate. After having been kept in a sterile featureless environment for one week, they were placed in the test chamber. Once again, all individuals exhibited a distinct preference for the substrate pattern upon which they were reared, as indicated by their initial choice and time spent on the substrate. This indicates a definitive retention capacity for specific physical cues in the habitat.

Discussion

The results of these experiments demonstrate that *K. pulchra* tadpoles can establish preferences for substrate patterns based on physical features present in the habitat where they emerge from the egg. These effects of early experience on habitat selection are shown in Table 1 and Fig. 2. In all groups, the majority of tadpoles chose the habitat pattern that they had been subjected to upon hatching over one which was unfamiliar to them. In addition, the animals spent a great deal more time on the substrate which resembled that of the rearing habitat. In addition to the relatively rapid acquisition of habitat preferences after hatching, these tadpoles demonstrate the capacity to retain these preferences even after periods of isolation from the substrate patterns, as well as through the developmental changes taking place during metamorphosis. Furthermore, as pointed out by Wiens (1970), the acquisition of this preference response does not appear to be characterized by a critical period during which the preference must be established as the older tadpoles established a preference as quickly as the younger animals. This suggests that the underlying mechanisms involved in the formation of preference responses are different from those which characterize imprinting behaviour. The importance of such critical periods in imprinting is discussed in detail by Bateson (1966), Bateson & Reese (1969), and Sluckin (1965).

Microhylids of the genus *Kaloula* are characterized by vertical pupils, palatine bones which form a toothed ridge across the palate, digits free or webbed, outer metatarsals which are united, precoracoids and omosternum absent, a cartilagenous sternum, and the diapophyses of the sacral vertebrae being slightly dilated (Boulenger 1890; Parker 1934; Porter 1972; Smith 1935). There are eight known species, three of which occur in the Malay Archipelago (Parker 1934). The Malayan painted frog, *K. pulchra,* ranges from Peninsular India and Sri Lanka, to Burma, southern China and the Malay Peninsula. Tadpoles used in this study were reared from adults orginally collect-

TABLE 1

INITIAL CHOICE OF SUBSTRATE PATTERNS MADE BY *Kaloula pulchra* TADPOLES REARED IN THREE EXPERIMENTAL HABITATS

Group	Rearing habitat pattern	Developmental stage	Number choosing per 30 tadpoles: Squares	(%)	Stripes	(%)	z – Value	
1	Sterile Environment	1	17	(56.6)	13	(43.3)	0.52	
		2	12	(40.0)	18	(60.0)	0.81	
		3	14	(46.6)	16	(53.3)	0.29	
		1	26	(86.6)	4	(12.3)	2.65	($P<.01$)
	Squares	2	21	(70.0)	9	(30.0)	2.09	($P<.01$)
2		3	24	(80.0)	6	(20.0)	2.48	($P<.01$)
		1	4	(12.3)	26	(86.6)	2.65	($P<.01$)
	Stripes	2	1	(03.3)	29	(96.6)	3.47	($P<.01$)
		3	3	(10.0)	27	(90.0)	2.84	($P<.01$)
		1	20	(66.6)	10	(33.3)	1.61	($P<.05$)
	Squares	2	21	(70.0)	9	(30.0)	1.98	($P<.05$)
3		3	24	(80.0)	6	(20.0)	2.48	($P<.01$)
		1	7	(23.3)	23	(76.6)	2.33	($P<.01$)
	Stripes	2	9	(30.0)	21	(70.0)	2.09	($P<.01$)
		3	5	(16.6)	25	(83.3)	2.37	($P<.01$)

ed in Sri Lanka. Adults of *K. pulchra* are generally fossorial in habit, and feed extensively at the surface on hymenopterous insects found in or near decaying vegetation (Porter 1972; Smith 1935).

The adaptive significance of learning a preference for a particular substrate pattern is of supreme importance to the survival of these tadpoles. The adult females normally deposit the eggs in a favourable environment thereby ensuring that the hatchling tadpoles will encounter physical cues from the spatial patterning of this habitat, learn to establish a preference for them, and maintain themselves in this habitat until their development is completed. Undoubtedly, the optimal habitat of a species is one which confers a certain degree of camouflage for the animal thereby making it more difficult to detect by its potential predators. Therefore, the survival capacity of the species is greatly increased by the ability to select and remain in the optimal environment. For example, tadpoles that were hatched in an aquatic environment characterized by slender, submergent branches, stems, grasses and algae which are basically linear objects that would project linear shadows on sandy substrates, would have a selective advantage if they were able to rapidly establish a preference for that substrate pattern and thus maintain themselves in a more favourable, cryptic environment. Furthermore, in rapidly flowing streams where tadpoles might be carried by currents, the ability to respond vigorously when confronted by an unfamilar substrate pattern would facilitate the animal in relocating its position to the more favourable habitat. The results of this experiment confirm this hypothesis. When *K. pulchra* tadpoles entered the area of the test chamber characterized by an unfamiliar substrate pattern they became extremely agitated and quickly swam back to the area provided with the pattern on which they had been reared. More frequently, the animals would stop at the boundary between the two substrate patterns and refuse to enter the unfamiliar area.

It is evident from the previous discussion that tadpoles of *K. pulchra* have the capacity to learn rapidly to respond in a positive manner to the substrate pattern on which they are reared, thereby establishing a habitat preference based on early experience. This suggests that the preference response for a particular habitat is not an instinctual and rigid behavioural act but rather a flexible response capable of a high degree of modification. Perhaps the potential to learn to respond selectively to a particular spatial patterning of the environment is innate, but the actual preference established is a function of the substrate pattern that the tadpoles first encounter upon hatching from the egg. This is the first demonstration of the effects of early experience on subsequent habitat selection by microhylid tadpoles, and is in general agreement with some of the findings reported for larval ranids (Heatwole 1962; Sexton *et al.* 1964; Wiens 1970, 1972) and salamanders (McKenzie & Storm 1970; Schneider 1968). In addition, once the preference for a particular habitat has been established, it is retained even over a period of isolation from the preferred substrate. Thus, the role of learning in the evolutionary success of anurans in general, may not be as diminutive a one as suggested by some previous investigators (Nobel 1931; Thorpe 1963).

References

AMBROSE, H. W. (1973): An experimental study of some factors affecting the spatial and temporal activity of *Microtis pennsylvanicus*. *J. Mammal.* *54*(1):79-110.

BARASH, D. P. (1973): Habitat utilization in three species of subalpine mammals. *J. Mammal.* *54*(1): 247-251.

BATESON, P. P. G. (1966): The characteristics and context of imprinting. *Biol. Rev.* *41*:177-220.

——————— & REESE, E. P. (1969): The reinforcing properties conspicuous stimuli in the imprinting situation. *Anim. Behav.* *17*:692-699.

BOULENGER, G. A. (1890): The Fauna of British India, including Ceylon and Burma: Reptilia and Batrachia. Thacker & Co., Bombay.

CAMERON, G. N. & RAINEY, D. (1972): Habitat selection by *Neotoma lepida* in the Mojave Desert. *J. Mammal.* *53*(2):251-266.

CHU, P. K. & MCCAIN, G. (1969): Discrimination learning and extinction in toads. *Psychon. Sci.* *14*:14-15.

GELUSO, K. N. (1971): Habitat distribution of *Peromyscus* in the Black Mesa region of Oklahoma. *J. Mammal.* *52*(3):605-607.

GOODMAN, D. E. & GOIN, C. (1970): The habitat of *Centrolene geckoideum* in Ecuador. *Herpetologica* *26*(2):276-278.

GOSNER, K. L. (1960): A simplified table for staging anuran embryos and larva with notes on identification. *Herpetologica* *14*(3):27-39.

HEATWOLE, H. (1961): Habitat selection and activity of the wood frog, *Rana sylvatica*. *Amer. Midl. Nat.* *66*:301-313.

——————— (1962): Environmental factors affecting local distribution and activity of the salamander, *Plethodon cinereus*. *Ecology* *43*:460-472.

HILDEN, O. (1965): Habitat selection in birds. *Ann. Zool. Fenn.* *2*:53-75.

KLOPFER, P. H. (1967): Stimulus preference and imprinting. *Science* *156*:1394-1396.

——————— & HAILMAN, J. P. (1965): Habitat selection in birds. *In*: Advances in the Study of Behaviour. D. S. Lehrman, R. A. Hinde & E. Shaw, eds. *1*:279-303, Academic Press, New York.

KUNTZ, A. (1923): The learning of a simple maze by the larva of *Ambystoma tigrinum*. *Univ. Iowa Studies in Nat. Hist.* *10*:27-35.

LACK, D. & VENABLES, L. S. (1939): The habitat distribution of woodland birds. *J. Anim. Ecol.* *8*:39-71.

MCKENZIE, D. S. & STORM, R. (1970): Patterns of habitat selection in the clouded salamander, *Aneides ferreus*. *Herpetologica* *26*(4):211-217.

MILLER, A. (1942): Habitat selection among higher vertebrates and its relation to interspecific variation. *Amer. Nat.* *76*:25-35.

MUNN, N. L. (1940): Learning experiments with larval frogs. *J. Comp. Physiol. Psychol.* *29*:97-108.

NOBLE, G. K. (1931): The Biology of the Amphibia. McGraw-Hill, New York.

PARKER, H. W. (1934): A Monograph of the Frogs of the Family Microhylidae. British Museum, London.

PORTER, K. R. (1972): Herpetology. Saunders, New York.

SARGENT, T. D. (1965): The role of early experience in the nest building of the zebra finch. *Auk* *82*:48-61.

SCHNEIDER, C. W. (1968): Avoidance learning and the response tendencies of the larval salamander, *Ambystoma punctatum* to photic stimulation. *Anim. Behav.* *16*(4):492-496.

SEXTON, O. J., HEATWOLE, H. & KNIGHT, D. (1964): Correlation of microdistribution of some Panamanian reptiles and amphibians with the structural organization of the habitat. *Carib. J. Sci.* *4*:261-295.

——————— & ORTLEB, E. (1966): Some cues used by leptodactylid frogs, *Engystomops pustulosus*, in selection of an oviposition site. *Copeia* (1966): 225-230.

SLUCKIN, W. (1965): Imprinting and early learning. Aldine, Chicago.

SMITH, M. A. (1935): The Fauna of British India including Ceylon and Burma: Reptilia and Amphibia. Vol. II. Taylor & Francis, London.

THORPE, W. H. (1963): Learning and instinct in animals. Harvard Univ. Press, Cambridge, Massachusetts.

WECKER, S. C. (1963): The role of early experience in habitat selection by the prairie deer mouse, *Peromyscus maniculatus bairdi*. *Ecol. Monogr.* *33*: 307-325.

WIENS, J. A. (1970): Effects of early experience on substrate pattern selection in *Rana aurora* tadpoles. *Copeia* (1970): 543-548.

——————— (1972): Anuran habitat selection: early experience and substrate selection in *Rana cascadae* tadpoles. *Anim. Behav.* *20*(2):218-221.